ENGLISH

EnteroPluri-*Test*

Identification system of Enterobacteriaceae and other gram negative, oxidase negative bacteria

DESCRIPTION

EnteroPluri-*Test* is a 12-sector system containing special culture media that permits identification of the *Enterobacteriaceae* and other gram negative, oxidase negative bacteria.
The system allows the simultaneous inoculation of all media present in the sectors and the execution of 15 biochemical reactions.
Microorganism is identified evaluating the colour change of the different culture media after 18-24 hours of incubation at 36 °C ± 1 °C and by a code numbe r obtained from biochemical reaction interpretation.

CONTENT OF THE PACKAGE

Each package contains 10 or 25 **EnteroPluri-*Test***, 1 Instruction sheet and 1 data chart for biochemical reaction results.

ITEMS NECESSARY BUT NOT INCLUDED IN THE PACKAGE

Kovac's Reagent	Cod. 80270
EnteroPluri-*Test* Codebook	Cod. 71709
Oxidase test sticks / swabs / discs	Cod. 88029 / 88003 / 88004
VP test EP	Cod. 80281

- Sundry microbiology laboratory materials

CONFIGURATION

The configuration of the system is shown in Table n°1.

Table n°1:

Sector	***BIOCHEMICAL REACTIONS***
Glucose/Gas	Glucose fermentation and gas production in anaerobiosis
Lysine	Lysine decarboxylation in anaerobiosis
Ornithine	Ornithine decarboxylation in anaerobiosis
H_2S/ Indole	Hydrogen sulphide production and indole test
Adonitol	Adonitol fermentation
Lactose	Lactose fermentation
Arabinose	Arabinose fermentation
Sorbitol	Sorbitol fermentation
VP	Acetoin production (Voges-Proskauer)
Dulcitol/PA	Dulcitol fermentation and phenylalanine deamination
Urea	Urea hydrolysis
Citrate	Citrate utilization

PRINCIPLE OF THE METHOD

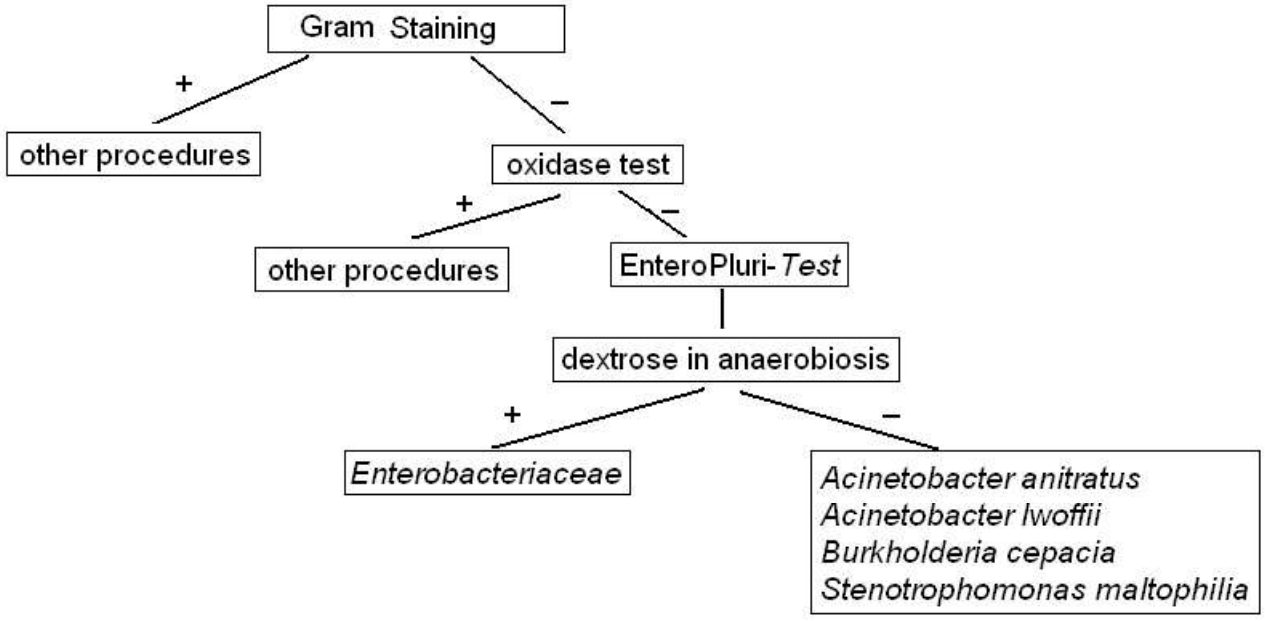
EnteroPluri-*Test* makes possible the identification of the *Enterobacteriaceae* and other gram negative, oxidase negative bacteria isolated from clinical and environmental samples.
The identification is based on biochemical tests performed on culture media containing specific substrates. The combination of positive and negative reactions allows to build up a code number that permits to identify bacteria by using the **Codebook.**

SAMPLE COLLECTION

EnteroPluri-*Test* is used for identification of gram negative, oxidase negative bacteria isolated in selective culture media for *Enterobacteriaceae* growth as: Mac Conkey Agar (MCA), Eosin Methylene Blue Agar (EMBA), Salmonella and Shigella Agar (SSA), Hektoen Enteric Agar (HEEA), or in not selective culture media.

TEST PROCEDURE

The microorganism to be identified should be recently isolated (18-24 hours): bacteria from cultures older than 48 hours can provide unreliable results.
Before inoculating the microorganism to be identified, it's compulsory to perform a gram staining and oxidase test on the microorganism. Only gram negative, oxidase negative bacteria should be inoculated on **EnteroPluri-*Test***. For the correct performace of both tests please consult appropriate bacteriology manuals.



- Pick up an **EnteroPluri-*Test*** system from the package and note identificative name of bacterial strain to submit to identification, date of test and other useful information.
- Remove both caps of the system. Using the tip of inoculating needle, placed under the blue cap, and without flambing, pick up a well isolated colony from a selective or non selective agar medium, without penetrating into the agar.
- Inoculate **EnteroPluri-*Test*** turning and withdrawing the needle throughout the sectors of the system.
- Reinsert the needle with a turning movement until the breakage notch; break the inoculating needle folding it in correspondence with the notch. The portion of the needle remaining inside the system keeps anaerobic conditions necessary for reactions of the sectors **Glucose/Gas**, **Lysine** and **Ornithine**.
- Use the broken portion of the needle, remained in the user hands, to punch the plastic film in correspondence of the holes of the sectors **Adonitol**, **Lactose**, **Arabinose**, **Sorbitol**, **VP**, **Dulcitol/PA**, **Urea**, **Citrate** in order to support aerobic growth.
- Scew again both caps and incubate **EnteroPluri-*Test*** at 36 ℃ ± 1 ℃ for 18-24 hours, putting it on its flat surface or vertically in a test-tube holder with the sector Glucose/Gas pointing upward.

INTERPRETATION OF RESULTS

At the end of incubation:

- Observe the change in colour of culture media in the different sectors and interpret results using the table n°2 and, in case, an **EnteroPluri-*Test*** not inoculated and kept at room temperature.

  NOTE: if there is no change in colour in the sector **Glucose/Gas** while in some other sectors there are chromatic changes, the microorganism under test does not belong to the family of *Enterobacteriaceae*. The **Codebook** also includes many codes of microorganisms that do not ferment glucose in anaerobiosis, but sometimes some additional biochemical reactions may be necessary for a correct identification of these nonfermenters.

- Record the obtained results on the enclosed data chart, except Indole test (sector **H_2S/Indole**) and Voges-Proskauer test (sector **VP**). Then perform Indole and Voges-Proskauer tests.

  Indole test
  Lay **EnteroPluri-*Test*** with its flat surface pointing upward and, by punching the plastic film, inject with a syringe 3 or 4 drops of Kovac's Reagent in the sector **H_2S/Indole.**
  The reaction is positive if a pink-red colour develops in the added reagent within 10-15 seconds.

  Voges-Proskauer test
  Lay **EnteroPluri-*Test*** with its flat surface pointing upward and, by punching the plastic film, inject with a syringe 3 drops of α-naphtol (Reagent 1) and 2 drops of potassium hydroxide (Reagent 2).
  The reaction is positive if a red colour develops within 20 minutes.

- Form the 5-digit code following the instructions provided in the paragraph **CODE NUMBER FORMING**. Identify the bacterium using the **Codebook.**

Table n°2:

Sector	***BIOCHEMICAL REACTIONS***	**Sector colour**	
		Positive reaction	**Negative reaction**
Glucose / Gas	Glucose fermentation	yellow	red
	Gas production	lifted wax	overlaying wax
Lysine	Lysine decarboxylation	violet	yellow
Ornithine	Ornithine decarboxylation	violet	yellow
H_2S / Indole	Hydrogen sulphide production	black-brown	beige
	Indole test	pink-red	colourless
Adonitol	Adonitol fermentation	yellow	red
Lactose	Lactose fermentation	yellow	red
Arabinose	Arabinose fermentation	yellow	red
Sorbitol	Sorbitol fermentation	yellow	red
VP	Acetoin production	red	colourless
Dulcitol/PA	Dulcitol fermentation	yellow	green
	Phenylalanine deamination	dark brown	green
Urea	Urea hydrolysis	purple	beige
Citrate	Citrate utilisation	blue	green

CODE NUMBER FORMING

1) The 15 biochemical tests are divided into 5 groups each containing 3 tests and each one is indicated with a positivity value of 4, 2,1.

- Value 4 : first test positive in each group (**Glucose**, **Ornithine**, **Adonitol**, **Sorbitol**, **PA**)
- Value 2 : second test positive in each group (**Gas**, **H_2S**, **Lactose**, **VP**, **Urea**)
- Value 1 : third test positive in each group (**Lysine**, **Indole**, **Arabinose**, **Dulcitol**, **Citrate**)
- Value 0 : every negative test

2) Adding the number of positive reactions in each group, it is obtainable a 5 digit code which, by the use of the **Codebook**, allows the identification of the microorganism under examination as in the following example.

	Group 1			**Group 2**			**Group 3**			**Group 4**			**Group 5**		
Test	**Glucose**	**Gas**	**Lysine**	**Ornithine**	**H_2S**	**Indole**	**Adonitol**	**Lactose**	**Arabinose**	**Sorbitol**	**VP**	**Dulcitol**	**PA**	**Urea**	**Citrate**
Positivity code	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1
Results	+	+	-	+	+	-	-	-	-	-	+	-	+	+	-
Code	4+2+0=6			4+2+0=6			0+0+0=0			0+2+0=2			4+2+0=6		
CODICE: 66026	IDENTIFICATION: *Proteus mirabilis*														

USER QUALITY CONTROL

Inoculate **EnteroPluri-*Test*** using the reference bacterial strains indicated in the table n°3.
For inoculation, incubation and reading please follow the instructions indicated in the paragraph **TEST PROCEDURE.**

Table n°3:

Microorganisms	**Glucose**	**Gas**	**Lysine**	**Ornithine**	**H_2S**	**Indole**	**Adonitol**	**Lactose**	**Arabinose**	**Sorbitol**	**VP**	**Dulcitol**	**PA**	**Urea**	**Citrate**	**Acceptable biocodes**
Escherichia coli ATCC 25922	+	+	+	+	-	+	-	+	+	+	-	-	-	-	-	75340
Proteus mirabilis ATCC 25933	+	+	-	+	+	-	-	-	-	-	-	-	+	+	+	66007
Klebsiella pneumoniae ATCC 13883	+	+	+	-	-	-	+	+	+	+	±	+	-	±	+	70773-70771 70753-70751
Salmonella typhimurium ATCC 14028	+	-	+	-	+	-	-	-	+	+	-	-	-	-	-	52140
Pseudomonas aeruginosa ATCC 27853	-	-	+	+	-	-	-	-	±	-	-	-	-	±	±	*

* *Pseudomonas aeruginosa* is oxidase positive, therefore it is not included in the **EnteroPluri-*Test* Codebook**

TABLE OF BIOCHEMICAL REACTIONS

Table n°4: Percentage of strains giving positive reactions with 18-24 h incubation at 36 °C ± 1 °C

			Gucose	Gas	Lysine	Ornithine	H_2S	Indole	Adonitol	Lactose	Arabinose	Sorbitol	Voges-Proskauer	Dulcitol	Phenylalanine	Urea	Citrate
Escherichieae		*Escherichia*	+ 100.0	+J 92.0	d 80.6	d 57.8	-K 4.0	+ 96.3	- 5.2	+J 91.6	+ 91.3	+/- 80.3	- 0.0	d 49.3	- 0.1	- 0.1	- 0.2
		Shigella	+ 100.0	-A 2.1	- 0.0	-/+B 20.0	- 0.0	-/+ 37.8	- 0.0	-B 0.3	+/- 67.8	-/+ 29.1	- 0.0	d 5.4	- 0.0	- 0.0	- 0.0
Edwardsiellae		*Edwardsiella*	+ 100.0	+ 99.4	+ 100.0	+ 99.0	+ 99.6	+ 99.0	- 0.0	- 0.0	+/- 10.7	- 0.2	- 0.0	- 0.0	- 0.0	- 0.0	- 0.0
Salmonelleae		*Salmonella*	+ 100.0	+C 91.9	+H 94.6	+I 92.7	+E 91.6	- 1.1	- 0.0	- 0.8	+/- 89.2	+ 94.1	- 0.0	dD 86.5	- 0.0	- 0.0	dF 80.1
		Arizona	+ 100.0	+ 99.7	+ 99.4	+ 100.0	+ 98.7	- 2.0	- 0.0	D 69.8	+ 99.1	+ 97.1	- 0.0	- 0.0	- 0.0	- 0.0	+ 96.8
	Citrobacter	*freundii*	+ 100.0	+ 91.4	- 0.0	d 17.2	+/- 81.6	- 6.7	- 0.0	d 39.3	+ 100.0	+ 98.2	- 0.0	d 59.8	- 0.0	dw 89.4	+ 90.4
		amalonaticus	+ 100.0	+ 97.0	- 0.0	+ 97.0	- 0.0	+ 99.0	- 0.0	+/- 70.0	+ 99.0	+ 97.0	- 0.0	-/+ 11.0	- 0.0	+/- 81.0	+ 94.0
		diversus	+ 100.0	+ 97.3	- 0.0	+ 99.8	- 0.0	+ 100.0	+ 100.0	d 40.3	+ 98.0	+ 98.2	- 0.0	+/- 52.2	- 0.0	dw 85.8	+ 99.7
Proteeae	*Proteus*	*vulgaris*	+ 100.0	+/-G 86.0	- 0.0	- 0.0	+ 95.0	+ 91.4	- 0.0	- 0.0	- 0.0	- 0.0	- 0.0	- 0.0	+ 100.0	+ 95.0	d 10.5
		mirabilis	+ 100.0	+G 96.0	- 0.0	+ 99.0	+ 94.5	- 3.2	- 0.0	- 2.0	- 0.0	- 0.0	-/+ 16.0	- 0.0	+ 99.6	+/- 89.3	+/- 58.7
	Morganella	*morganii*	+ 100.0	+/-G 86.0	- 0.0	+ 97.0	- 0.0	+ 99.5	- 0.0	- 0.0	- 0.0	- 0.0	- 0.0	- 0.0	+ 95.0	+ 97.1	- 0.0
	Providencia	*alcalifaciens*	+ 100.0	dG 85.2	- 0.0	- 1.2	- 0.0	+ 99.4	+ 94.3	- 0.3	- 0.7	- 0.6	- 0.0	- 0.0	+ 97.4	- 0.0	+ 97.9
		stuartii	+ 100.0	- 0.0	- 0.0	- 0.0	- 0.0	+ 98.6	-/+ 12.4	- 3.6	- 4.0	- 3.4	- 0.0	- 0.0	+ 94.5	-/+ 20.0	+ 93.7
		rettgeri	+ 100.0	-/+G 12.2	- 0.0	- 0.0	- 0.0	+ 95.9	+ 99.0	d 10.0	- 0.0	- 1.0	- 0.0	- 0.0	+ 98.0	+ 100.0	+ 96.0
Klebsielleae	*Enterobacter*	*cloacae*	+ 100.0	+ 99.3	- 0.0	+ 93.7	- 0.0	- 0.0	-/+ 28.0	+/- 94.0	+ 99.4	+ 100.0	+ 100.0	d 15.2	- 0.0	-/+ 74.6	+ 98.9
		sakazakii	+ 100.0	+ 97.0	- 0.0	+ 97.0	- 0.0	-/+ 16.0	- 0.0	+ 100.0	+ 100.0	- 0.0	+ 97.0	- 6.0	- 0.0	- 0.0	+ 94.0
		gergoviae	+ 100.0	+ 93.0	+/- 64.0	+ 100.0	- 0.0	- 0.0	- 0.0	-/+ 42.0	+ 100.0	- 0.0	+ 100.0	- 0.0	- 0.0	+ 100.0	+ 96.0
		aerogenes	+ 100.0	+ 95.9	+ 97.5	+ 95.9	- 0.0	- 0.8	+ 97.5	+ 92.5	+ 100.0	+ 98.3	+ 100.0	- 4.1	- 0.0	- 0.0	+ 92.6
	Pantoea	*agglomerans*	+ 100.0	-/+ 24.1	- 0.0	- 0.0	- 0.0	-/+ 19.7	- 7.5	d 52.9	+ 97.5	d 26.3	+/- 64.8	d 12.9	-/+ 27.6	d 34.1	d 84.2
	Hafnia	*alvei*	+ 100.0	+ 98.9	+ 99.6	+ 98.6	- 0.0	- 0.0	- 0.0	d 2.8	+ 99.3	- 0.0	+/- 65.0	- 2.4	- 0.0	- 3.0	d 5.6
	Serratia	*marcescens*	+ 100.0	+/-G 52.6	+ 99.6	+ 99.6	- 0.0	-w 0.1	-/+ 56.0	- 1.3	- 0.0	+ 99.1	+ 98.7	- 0.0	- 0.0	dw 39.7	+ 97.6
		liquefaciens	+ 100.0	d 72.5	+/- 64.2	+ 100.0	- 0.0	-w 1.8	- 8.3	d 15.6	+ 97.3	+ 97.3	-/+ 49.5	- 0.0	- 0.9	dw 3.7	+ 93.6
		rubidaea	+ 100.0	dG 35.0	+/- 61.0	- 0.0	- 0.0	-w 2.0	+/- 88.0	+ 100.0	+ 100.0	- 8.0	+ 92.0	- 0.0	- 0.0	dw 4.0	+/- 88.0
	Klebsiella	*pneumoniae*	+ 100.0	+ 96.0	+ 97.2	- 0.0	- 0.0	- 0.0	+/- 89.0	+ 98.7	+ 99.9	+ 99.4	+ 93.7	-/+ 33.0	- 0.0	+ 95.4	+ 96.8
		oxytoca	+ 100.0	+ 96.0	+ 97.2	- 0.0	- 0.0	+ 100.0	+/- 89.0	+ 98.7	+ 100.0	+ 98.0	+ 93.7	-/+ 33.0	- 0.0	+ 95.4	+ 96.8
		ozaenae	+ 100.0	d 55.0	-/+ 35.8	- 1.0	- 0.0	- 0.0	+ 91.8	d 26.2	+ 100.0	+/- 78.0	- 0.0	- 0.0	- 0.0	d 14.8	d 28.1
		rhinoscleromatis	+ 100.0	- 0.0	- 0.0	- 0.0	- 0.0	- 0.0	+ 98.0	d 6.0	+ 100.0	+ 98.0	- 0.0	- 0.0	- 0.0	- 0.0	- 0.0
Yersineae	*Yersinia*	*enterocolitica*	+ 100.0	- 0.0	- 0.0	+ 90.7	- 0.0	-/+ 26.7	- 0.0	- 0.0	+ 98.7	+ 98.7	- 0.1	- 0.0	- 0.0	+ 90.7	- 0.0
		pseudotuberculosis	+ 100.0	- 0.0	- 0.0	- 0.0	- 0.0	- 0.0	- 0.0	- 0.0	+/- 55.0	- 0.0	- 0.0	- 0.0	- 0.0	+ 100.0	- 0.0

+	Positive	**D**	*S.typhi*, *S. cholerae-suis, S.enteritidis* bioserotypes *paratyphi* A and *pullorum,* and a few others do not ferment dulcitol promptly.
-	Negative		
+/-	Mostly positive	**E**	*S.enteritidis* bioserotypes *paratyphi* A and some rare biotypes may be H_2S-negative
-/+	Mostly negative	**F**	*S.typhi*, *S.enteritidis* bioserotypes *paratyphi* A and some rare biotypes are citrate-negative. *S.cholerae-suis* is usually delayed positive.
d	Different biochemical types	**G**	*Serratia, Proteus* and *Providencia alcalifaciens* develop a little quantity of gas. Their gas production bay be not evident.
w	Weak reaction	**H**	*S.enteritidis* bioserotype *parathyphi* A is lysine–negative.
A	Certain biotypes of *S.flexneri* form gas	**I**	*S.typhi and S.gallinarum* are ornithine-negative
B	*S.sonnei* strain usually ferment lactose very slowly	**J**	The *Alkalescens-Dispar* (A-D) group is included as a biotype of *E.coli.* Members of the A-D group are generally nonmotile, lactose-negative and do not form gas.
C	*S.typhi* and *S.gallinarum* are anaerogenic	**K**	An occasional strain may produce hydrogen sulfide.

FACTORS THAT MAY INVALIDATE THE RESULTS

- Use of mixed cultures.
- Application of the method to bacteria not belonging to the family of *Enterobacteriaceae* or to non gram negative, oxidase negative bacteria.
- Use of expired systems.
- Test procedure different from the one suggested.

PRECAUTION

The product, **EnteroPluri-*Test***, cannot be classified as hazardous under current legislation and does not contain harmful substances in concentrations ≥1%. It therefore does not require a Safety Data Sheet to be available. **EnteroPluri-*Test*** is a disposable device to be used only for *in vitro* diagnostic use*;* it is intended for use in a professional environment and should be used in laboratory by properly trained personnel, using approved asepsis and safety methods for handling pathogenic agents.

STORAGE

Store at 2-8 ℃ away from light. In such conditions , the product will remain valid until the expiry date indicated on the label. Do not use beyond that date. Eliminate without using them if there are signs of deterioration.

DISPOSAL OF USED MATERIAL

After use, **EnteroPluri-*Test*** should be decontaminated and disposed off in accordance with the techniques used in the laboratory for decontamination and disposal of potentially infected material.

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- EnteroPluri-*Test* Archivio Liofilchem, Marzo 2005.

PRESENTATION

Product	Code	Package
EnteroPluri-*Test*	78618	10 *test*
	78619	25 *test*

TABLE OF SYMBOLS

IVD *In Vitro* Diagnostic Medical Device	Do not reuse	Manufacturer	Contains sufficient for <n> tests	Temperature limitation
REF Catalogue number	Fragile, handle with care	Use by	Caution, consult accompanying documents	LOT Batch code
Store away from light				

Rev.2 / 08.04.2005

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